Introduction

The work included in this thesis, entitled "Foraging and Nest-Building Behavior of Indian Cliff Swallow, *Hirundo fluvicola* (Blyth, 1855)" has been divided in to six chapters. Chapter-I contains a review of literature on behavioral ecology of swallows. It also includes the *prélude* and objectives of the thesis. Chapter-II concerns with the annual variability in the pattern of roosting behavior and activity duration in Indian cliff swallow, *Hirundo fluvicola* as function of season and annual time scale. Chapter-III includes the daytime nest visitation activity rhythm in Indian cliff swallow, *Hirundo fluvicola*. Chapter-IV contains the ethology of nidification and morphology of the nests in Indian cliff swallow, *Hirundo fluvicola*. Chapter-V contains the mechano-physical properties and chemical components of mud used in the nests of Indian cliff swallow, *Hirundo fluvicola*. Chapter-VI Summary, conclusion and recommendations deals with the summary of each of the previous chapters. This also includes conclusions and recommendations those are emanated from this doctoral dissertation.

Chapter-I: Review of literature

Swallows represent the most accepted exemplar for behavioral and ecological research (Brown and Brown 1991; Winkler and Sheldon 1993; Turner and Rose 1989). The swallows have an evolutionarily slender, streamlined body with elongated edged wings, which complements immense mobility, pertinacity, and facilitate in recurrent period of gliding adapted for hunting insects while on the wing (Turner 2004). The sexes exhibit partial or no sexual dimorphism, except for longer tail feathers in the adult males (Møller 1992). The family exploits an extensive dimension of habitats. The nests and nesting sites varies among different species of swallow. Numerous species have expanded their range of habitation by inhabiting modified human surroundings consisting of agricultural land, bridges, culverts and urban locality (Brown and Brown 1986; Turner 2004).

Night roosts play an important role in avian biology, because birds spend a major amount of their time in their roost or nest during the night (Woltmann 2004). The benefits associated with communal roosting are chiefly amplified acquaintance on foraging efficiency (Ward and Zahavi 1973), reduction in thermoregulation costs (Hatchwell et al. 2009), and protection from predators (Krause and Ruxton 2002). The daily roosting time of birds in relation to sunrise and sunset time may vary in accordance with the changing environmental conditions and day length (Reebs 1986). The initiation and cessation of activity time can be considered as reflections of the endogenous clock (Chandrashekaran et al. 1983). In birds, time allotment to various activities could be influenced by numerous features, such as habitat (Rave and Baldassarre 1989), season & time of the

day (Pietz 1986), sex & age (Sullivan 1988), and communal systematization (Caraco 1979). Many field ornithologists have observed and found the occurrence of daily and seasonal activities – most of them are under the control of endogenous clocks, in birds (Wikelski et al. 2008; Helm et al. 2009). The adaptive advantage, which is bestowed by endogenous rhythms allow the organism to regulate its physiology and behavior in anticipation of forthcoming alterations in the environment (Goymann et al. 2012). Birds belonging to different taxa, exhibit significant variations in their nesting ecology, such as selection of the nesting habitat, materials used for the construction of nest, building pattern, shape, size and structure of nest (Hansell 2000). In many bird species, selection of suitable nesting site may be a significant predictor for its reproductive success (Tuomenpuro 1991). Swallows construct exclusively mud nests, which are attached to the vertical surface. Both the members of each pair contribute to the nest construction (Keith et al. 1992). In all species of Hirundinidae, except the members of the martin clade, nest completion time is comparable (Asbirk 1976). Selection for the nest lining material may be primarily based on the mechanical and physical properties (Heenan and Seymour 2011); however, the insulative properties can also be attributed to the nest lining (Surgey et al. 2012). According to Rowley (1969), about 5% of the avian species build nest using mud as a building material. Design and construction of mud nests are influenced by many properties of the mud, such as adhesion, cohesion, strength, workability, shrinkage, and swelling (Kilgore and Knudsen 1977). The composition of soil is a reflection of the following properties, such as shear strength, liquid limit (LL), and plastic limit (PL) (Punmia 1988). The swiftlets use saliva to bind materials together for nest construction and attach them to the vertical walls of in-land or sea-side caves (Sankaran 2001). The nests of barn swallow *Hirundo rustica* and cliff swallow *Petrochelidon fluvicola*, had protein and carbohydrate in it and the mineral content of nests was slightly higher than that of the control soil (Chaya et al. 2013).

The data included in this thesis will stimulate others to initiate behavioral field studies on avifauna. The study of various spectra in the pattern of daily and seasonal activities is paramount as it reflects the abilities of the birds to anticipate forthcoming changes in the environment. This cognitive ability of judging time might have been evolved through ages and resulted in the form of expression of rhythms in hundreds of physiological and behavioral variables. Systematic study of the nest sites, nest construction behavior, nesting materials and the phenology of the nest is relevant in tracing the life history, and ecology of the species; therefore, such studies have an important bearing on the development of strategies for its conservation. Information gathered in this study will be of immense value while assessing the abundance, vulnerability of the species and inclination in the demographic trend before actual decline in the population density occurs.

Chapter-II: Annual variability in roosting behavior and activity in Indian cliff swallow, *Hirundo fluvicola*: proximate factors and season

(Part accepted in *Biological Rhythm Research*, 2014)

This chapter deals with the study of pattern of roosting behavior and activity duration (= foraging behavior) in Indian cliff swallow, *Hirundo fluvicola* with respect to various environmental factors and season on an annual time scale. The nesting site selected for the study is located at 21˚15'07.54"N latitude; 81˚32'30.65"E longitude in the Raipur region of Chhattisgarh State. The site is located on the left flank of the existing bridge over the river Kharoon (a subsidiary feeder of the river Mahanadi) on the Raipur-Bhilai highway. The study was conducted along a longitudinal time scale from August 2009 to October 2011. The exit and entry timings of the first and the last birds, respectively, were monitored for eight consecutive days every month. Results indicated that the irrespective of seasons, the first bird emerged from the roost roughly 30 minutes before the local sunrise time. Similarly, the last bird entered to the roost about 30 minutes after the sunset. Exit time was found to be significantly associated with sunrise time, morning temperature, morning humidity and morning light intensity; however morning phase angle was significantly associated with sunrise time only. The factor 'season' produced a significant effect on the exit time. The first bird exited earliest in summer followed by rainy and winter seasons. The entry time was found to be significantly associated with sunset time, evening temperature and evening light intensity; however, corresponding phase angle was significantly associated with sunset time and evening light intensity. The factor 'season' produced significant effects on the entry time as well as corresponding phase angle. The entry of the last bird to the roost was the earliest during winter season followed by rainy and summer seasons. Further, a statistically significant circannual rhythm in activity duration was obtained with a peak located on June 22. Results of MLR analysis indicated that the contribution of the factor 'day length' was the maximum (96%). Projection of the seasonal (rainy, winter & summer) data set upon the factorial plane 1-2 based on principal component analysis (PCA) is illustrated in Fig. 2.1. The PCA depicted that the first two axes alone accounted for 80% of the total inertia.

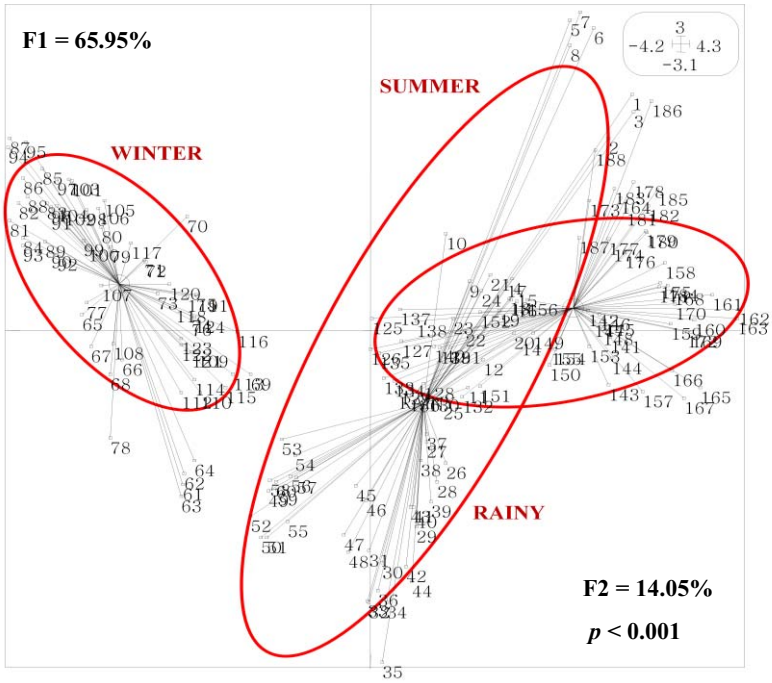


Figure 2.1 Simple PCA on the activity of Indian cliff swallow, *Hirundo fluvicola* based on nine variables during all seasons (rainy, winter and summer), all sites, and all data at a time

We conclude that the roosting behavior and activity duration (= foraging behavior) of Indian cliff swallow are affected and modulated by the environmental factors and seasons in this part of the Indian sub-continent. The PCA data also supports the findings on activity duration with reference to season. Activity duration exhibited significant circannual rhythm with a peak in the month of June over the seasons, which match with the breeding season and the abundance of insect. The day length appears to be one of the strongest predictors of activity duration on an annual time scale. The use of this data will provide new opportunities to study bird behavior in their free-living environmental conditions. Future studies can be extended experimentally to clarify the cost and benefit of communal roosting and to monitor temporal and spatial differences in roosting time at sufficiently large scale on a regional basis.

Chapter-III: Daytime nest visitation activity rhythm in Indian cliff swallow, *Hirundo fluvicola*

We studied the nest visitation activity pattern (exit and entry) of the Indian cliff swallow for two consecutive days under natural long photoperiod (May, June and July, 2011), and natural short photoperiod (November, December 2011 and January 2012). Results demonstrate that the bimodality for daytime nest visitation activity varied as function of photoperiod. It was found that during the long days the daytime nest visitation activity was less defined (multimodality; Fig. 3.1 and expanded throughout the day,

whereas during the short days activity was more precise (bimodality; Fig. 3.2) and was of shorter duration.

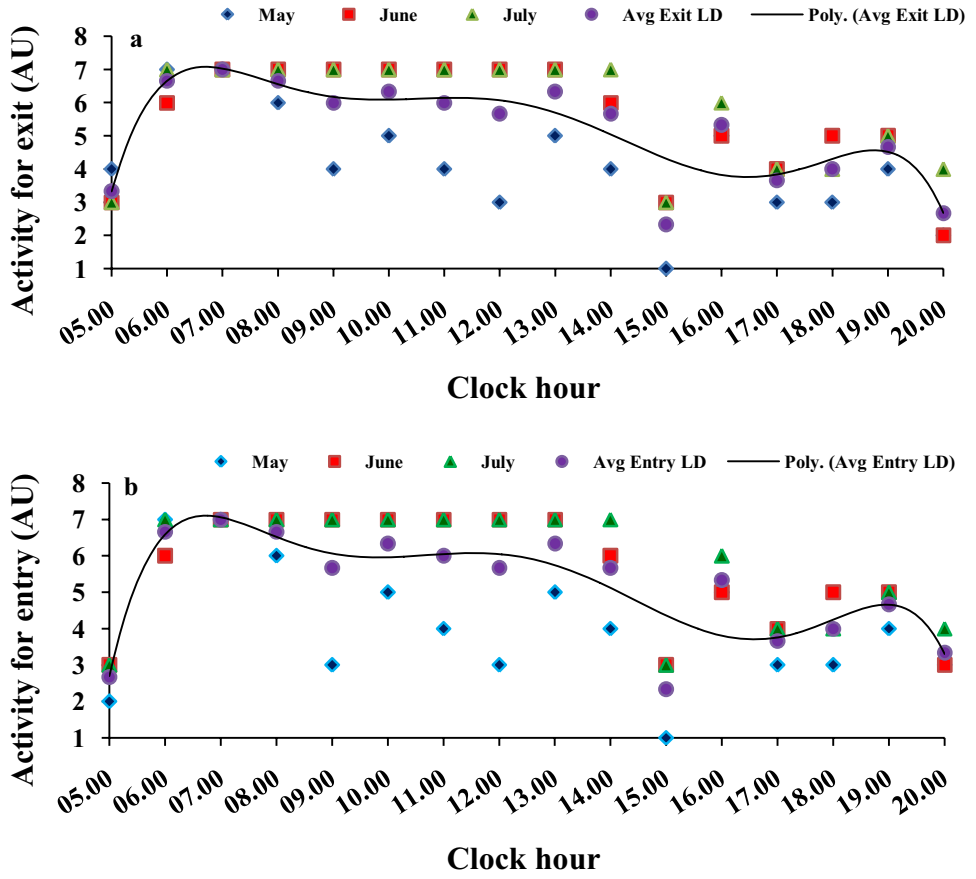
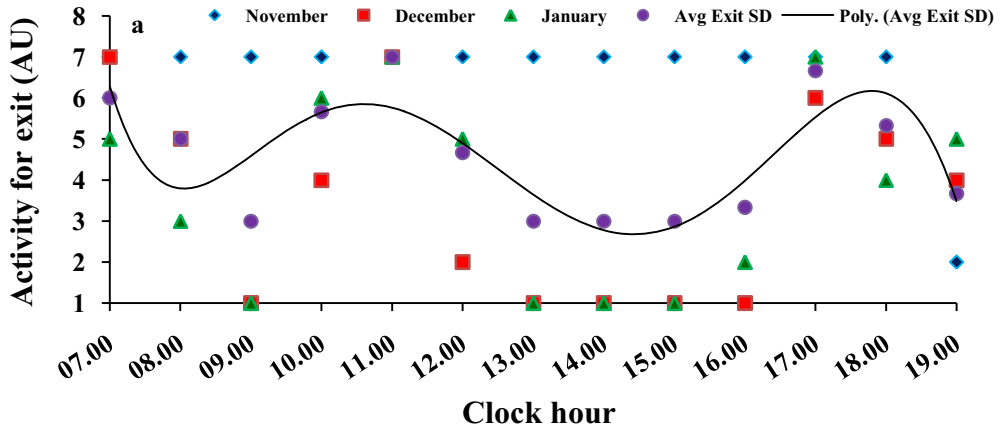


Figure 3.1 Multimodality pattern in daytime nest visitation based on raw data for exit (a) and entry (b) during long days



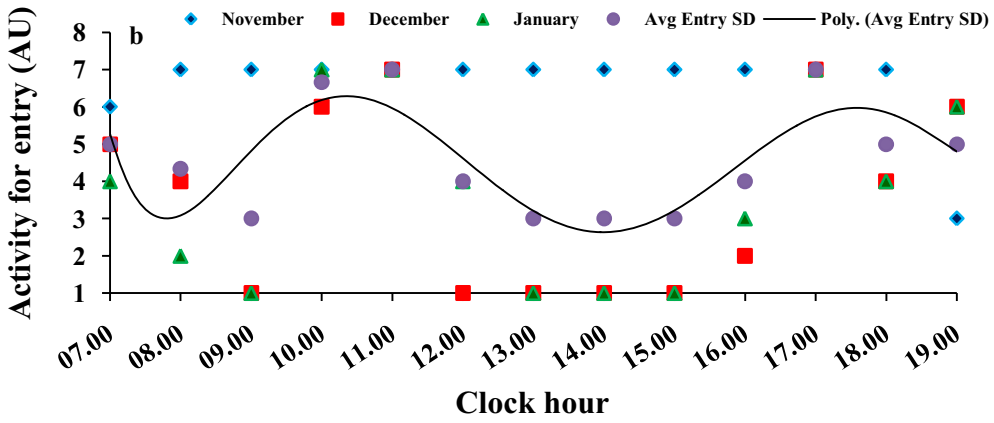


Figure 3.2 Bimodality pattern in daytime nest visitation based on raw data for exit (a) and entry (b) during short days

The factor 'time of the day' produced significant effect on the rate of daytime nest visitation of birds with respect of frequency. The overall nest visitation rate was always higher during the long days as compared to the time slots during short days. Further, the factors 'photoperiod' and 'time of the day' and their interaction produced significant effects on daytime pattern of nest visitation rate. A statistically significant circadian rhythm was validated in daytime nest visitation activity of Indian cliff swallow in all studied months and at group level during both long and short days. The harmonic means of the circadian amplitudes of the daytime nest visitation activity rhythm was significantly higher for both exit and entry during long days as compared to short days. The 24-h acrophase of activity occurred earlier during long days than short days.

In conclusion, the present study reported a statistically significant circadian rhythm in daytime nest visitation activity during both long and short days. A shift from a distinct bimodal daytime nest visitation activity pattern observed in short days to a platykurtic pattern in long days might be offering a prolonged duration to the birds for foraging and for procuring nesting materials for construction. It will be worthwhile to employ more sophisticated electronic tools, such as IR sensors at the nest sites to study the spectra of wave forms present in the nest visitation activity rhythm in this and other similar species.

Chapter-IV: Ethology of nidification and morphology of the nests in Indian cliff swallow, *Hirundo fluvicola*

In Indian cliff swallow, the main constituent of the nesting material is mud with fragments of grasses in between. The birds collected the mud from the road side puddles and the exposed river bank for nest construction. The entire nest construction process was divided into seven phases (Fig. 4.1 a,b). The entire process of nest building lasted between 19-25 days. The factor 'day' produced significant effect on phases of the nest

construction. Phase 5 (Bowl-shaped nest) takes significantly more days than the others for completion. ANOVA results indicated that the factors 'phase' and 'visit' and their interaction produced significant effects on the time (sec) spent inside and outside the nest. Further, with respect to frequency of visits inside and outside the nest, results indicated that the 'phase' is an important factor that produced significant effect on frequency of visits. Ratio of the time spent by the birds in the nests was found to be higher during late latter phases of the nest construction. The structure of the nest showed variations in length of the entrance tunnel, outer entrance tunnel perimeter, upper attachment length, lower attachment length and inside depth at all the sites namely, left arch, center of arch and right arch.

Figure 4.1 Nest construction from the initiation (a) to cessation (b)

To conclude, Indian cliff swallows are colonial mud nesters and construct their nests on the walls beneath the arch-shaped old bridges. Arbitrarily seven phases can be demarcated in the process of nidification. The variability in nest morphometry could be attributed to the nest location on the same nesting site of the arch. Several factors, such as time and variation in climatic conditions may contribute to overall variation in the time duration of nest construction and nest size of Indian cliff swallow. The results of the present study, however, substantiates that the species demonstrates remarkable consistency in the nest construction duration.

Chapter-V: Mechano-physical properties and chemical components of mud used in the nests of Indian cliff swallow, *Hirundo fluvicola*

In this study complete mechano-physical properties of the mud, used in the nests, were examined and its chemical components were determined as function of nest locations in the arched underneath of the bridge. The mechanical properties, namely liquid limit (LL), plastic limit (PL) and plasticity index (PI) of the nest soil was 27.13%,

21.33% and 6.33%, respectively. The factor 'arch location' produced significant effects on LL and PI. The LL and PI were significantly higher in right arch than the others. The factor 'type of soil' also produced significant effects on all three mechanical properties. LL and PI were significantly higher in black soil, whereas PL was higher in nest soil. The nests were composed mainly of medium sand (38.91%) followed by fine sand (31.94%), silt & clay (25.50%), coarse sand (2.87%), and gravel (0.73%). Significant effects of the factors, namely 'arch location' and 'type of soil' were validated on most of the physical components, namely medium sand, silt & clay, coarse sand and gravel of mud. Further, chemical components of mud did not vary as function of 'arch location' but varied significantly as function of 'type of soil.'

In summary, variations were observed in the liquid limit and plastic limit of nest soil at all the locations of the arch and also among the red, black and nest soil. There was variation in the amount of soil particles in the nest sample at different locations of the arch due to the dissimilar structural architecture of the nest at different sites and also in the texture of the sand particles in the red soil, black and nest soil. The chemical content of all the soils varied in their composition depending upon the soil type and texture. The findings of the present study shed light on the structural details of the mud nests apropos the sources of the mud. Similar investigation is desirable for other mud nesting species of birds.

General Conclusion and Recommendation

Indian cliff swallows are aerial insectivores and act as biocontrol agents to help in the eradication of harmful insects affecting humans, animals and farmland crops. However, the extensive use of pesticides and consequent reduction of insect population may be a threat to the population of swallows. This species build characteristic mud nests that exhibits enormous plasticity to cope with adverse conditions of its immediate environment. The principles that make these mud nests so much resilient to vagaries of nature can be extrapolated while building earth based shelters/homes for human use. Future studies can be planned to identify the important aspects, like distribution of insects, climate change, habitat destruction, and ecological factors, which pose a threat to the survival of the species. For conservation of a particular species collection of detailed information about its different behavior, nesting ecology and the factors that affect its breeding and nesting success, along the temporal and spatial planes is a must. Knowledge of life history traits taken together with the data on nesting and breeding behavior and ecology of avifauna can also provide information on demographic trends and warn the masses of their predicaments about the existence of the species before the decline in their population density really transpires.

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Day length and evening temperature predict circannual variation in activity duration of the colony of the Indian cliff swallow, Hirundo fluvicola

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Day length and evening temperature predict circannual variation in activity duration of the colony of the Indian cliff swallow, *Hirundo fluvicola*

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In avian species, circannual rhythms, in a number of biological variables, including locomotor activity, have been studied under both laboratory and natural environmental conditions. However, information on circannual rhythm in daily activity duration of Indian cliff swallow (*Hirundo fluvicola*) is not available. It is a communal mud nesting, non-migratory species and inhabits low under arch bridges. Although it figures in the IUCN Red List under the least concerned category, it is important to study its behavioral ecology that may be of utmost importance from conservation stand point. In the present investigation, we examined the circannual rhythm in daily activity duration in this species at a communal roosting site under Kharoon river bridge on the Raipur–Bhilai highway (21°15′07.54″N; 81°32′30.65″E), Raipur, India, for eight consecutive days, every month from August 2009 to October 2011. On each study day, observations were made in two sessions, morning and evening. We monitored and recorded emergence time of the first bird from the nest in the morning and entry time of the last bird to the nest in the evening. We also recorded the ambient temperature and humidity of the study site simultaneously. Average daily activity duration (the time difference between the first bird's emergence time and the last bird's entry time from/to the nest, respectively) was computed for each month. A significant circannual rhythm in activity duration was validated. The peak of activity rhythm occurred on 22nd June with a spread between 19th June and 25th June. The activity duration was the shortest in winter and the longest in summer. The daily activity duration was positively correlated to the day length, sunset time, and morning and evening temperatures; whereas negatively correlated with the sunrise time, and morning and evening humidity. The multiple linear regression models suggest that day length alone explains 96% of the total variance in activity duration, whereas only 0.5% variance was attributable to evening temperature. We conclude that the day length is the strongest predictor of circannual variation in daily activity duration of the colony of Indian cliff swallow; whereas the evening temperature has very little effect. Further, we recommend that comparable studies under natural conditions might be very helpful to explore the effect of environmental cues on other intriguing behavioral decisions made by this and other avian species.

Keywords: *Hirundo fluvicola*; activity duration; circannual rhythm; day length; environmental factors; season

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Introduction

A biological rhythm with an endogenous period close to a year is called a "circannual rhythm" and is governed by a purported circannual clock that regulates physiological

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and behavioral events along with longitudinal annual time scale (Gwinner 1996; Johnsson 2008). The endogenous circannual clock ensures a stable phase relationship between internal and external oscillations (Saunders 2002; Dunlap et al. 2004; Aparicio et al. 2014).

Birds, being the most noticeable group, are often used as an indicator of variations in the environment. Their abilities to anticipate periodic environmental cues are well recognized (Jonzén et al. 2007; McNamara & Houston 2008; Wingfield et al. 2008). Birds adjust their daily activities receiving inputs from various external stimuli, such as day length, light intensity, temperature, humidity, availability of food, and predators. Apart from day length and light intensity (Aschoff & Wever 1962; Gwinner 1975), the daily activity duration in birds may also depend upon number of other factors, namely weather conditions (Lopez-Calleja & Bozinovic 2003; Tieleman et al. 2003), season (Martínez 2000; Dixit & Singh 2011; Goymann et al. 2012), habitat type (Eberhardt et al. 1989; Morton et al. 1989; Rave & Baldassarre 1989), gender (Arshad & Zakaria 2009), age (Beveridge & Deag 1987; Sullivan 1988), and communal hierarchy (Pulliam 1973; Caraco 1979). Notwithstanding our knowledge about the role of these factors, the temporal pattern in daily activity duration differs among species, because we have very little information about the role of non-photic cues in the variability in the seasonal responses from one species to another (Gwinner & Scheuerlein 1998; Scheuerlein & Gwinner 2002). It is presumed that non-photic cues might be working as complementary and modifying factors (Hau et al. 2004; Helm et al. 2006) for the day length and light intensity induced harmonization of seasonal periodicities.

Information is scanty on the behavioral ecology of this species that figures in IUCN Red List under the least concerned category. Notwithstanding its status, it is important to study its behavioral ecology that may be of utmost importance from conservation standpoint.

In animals, the locomotor activity has been identified as one of the important characteristics to reveal the seasonal variability in phase association between activity (internal) and time cues (external) in both captive and free living conditions (Marimuthu 1984). For the purpose of documentation, the initiation and end of roosting time in birds can be referred as activity time that can be applied to study the various parameters of endogenous rhythm (Chandrashekaran et al. 1983). Measuring the energy expenditure and daily activities of birds in the natural environment is important in quantitative assessment and understanding of varied characteristics; for instance, approach for feeding, struggle for resources, or contribution by the parents (Kacelnick & Houston 1984; Goldstein 1984). It has been conjectured that seasonal cycle of birds with respect to reproduction when harmonized with temperature, precipitation and amount of food will have better survival chances than those species that organize their seasonal cycle with an inflexible *zeitgeber*, like day length (Carey 2009).

How do the environmental factors in the subtropical region that usually act as *zeitgebers* to harmonize the intrinsic cycles to real time in Indian cliff swallow is unclear? Our purpose was to investigate how day length and ambient environmental variables interact in a subtropical environment to act as determinants of activity duration in this species along the longitudinal temporal scales of about two years and over different seasons. Knowledge of activity patterns in this species will help us to understand avian ecology, to establish suitable survey methodology, and eventually to develop various methods to manage this endemic species. These data may also prove useful for utilizing the qualitative ideas to a quantitative framework for protection of biodiversity, unraveling various aspects of the ecology and conservation biology of this species.

Therefore, in the present investigation, we examined the circannual rhythm in daily activity duration in Indian cliff swallow at a communal roosting site.

Materials and methods

Nesting site

The nesting site of a huge colony of this species was located underneath the arches of a discarded bridge in the left flank of the currently used Kharoon river bridge on the Raipur–Bhilai highway (climate – tropical; location 21°15′07.54″N; 81°32′30.65″E), Chhattisgarh, India (Figure 1(a) and (b)). The discarded bridge is about 100 years old with an average height of about six meters. The height of the bridge from the ground level of the river bed was measured using Ultrasonic Distance Meter, W/LASER Point (CB–1005). The depth of the water body below the bridge always varies between 1.5 and 2.00 meters. However, during rainy season, water level increases, and if heavy rain persists, entire nesting colony gets washed away due to flood in the river. The nesting site is surrounded by agricultural fields and patches of green forest.

Data sampling

The study on the activity duration was conducted along a longitudinal time scale from August 2009 to October 2011. The exit time of the first bird and entry time of the last bird from/to the roosting site were monitored for eight consecutive days, every month, for over a period of twenty-seven months, excluding three months (August 2010, October 2010, and July 2011) and four days in July 2010, as the nests got washed off due to flood in the river. During the study period, we visited the nesting site every month in the morning and evening before the bird emerged and entered from/to their nest. The observations were made in the morning at least one hour before the expected exit time of the first bird, and the session was completed half an hour after the last bird emerged from the nest. Similarly, in the evening, observations were made one hour before the expected entry time of the last bird and terminated half an hour after the last bird entered the nest. The time of exit of the first bird from the nest was regarded as the initiation of activity time and entry of the last bird to the nest as the termination of activity time of the colony. The time duration between the initiation (exit of the first bird) and end of activity (entry of the last bird) was termed as "activity duration" and

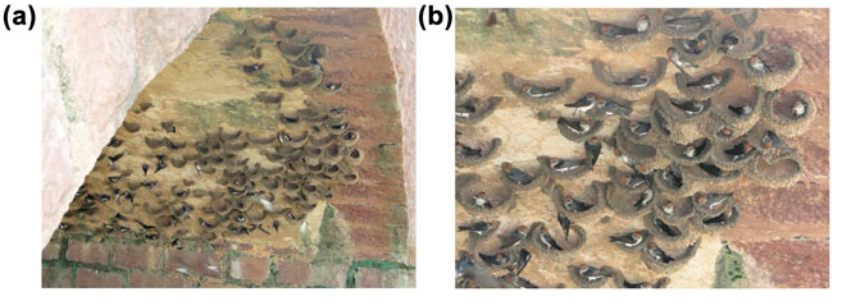

Figure 1. (Colour online) (a) A colonial nesting site of Indian cliff swallow, *Hirundo fluvicola*, located under one of the arches of the bridge; (b) a closer view of the nests and the birds.

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was expressed in hours. All observations for activity duration were carried out safely from a vantage point just above the arch, where the maximum number of communal nests was located. The annual time scale was demarcated into three seasons, namely rainy (monsoon), winter, and summer, in order to ascertain the effects of the factor, "season" on the duration of activity of the colony.

Environmental variables

Information about sunrise, sunset, and day length was obtained from the Raipur Meteorological Department. In addition, temperature and humidity were recorded in the morning and evening at the nesting site during the entire period of the study. The temperature and humidity were measured using a digital thermo-hygrometer (Pacer® TH 402). The descriptive statistics depicting averages and range of the environmental variables along the longitudinal time scale is illustrated in Table 1.

Statistical analyses

The data for activity duration were computed for each month and were subjected to Cosinor rhythmometry (Nelson et al. 1979; Gupta & Pati 1992) for validation of circannual rhythm. The circannual rhythm was characterized by three parameters, such as Mesor (*M*, rhythm-adjusted mean), the amplitude (*A*, half of the difference between minimum and maximum in the fitted cosine function), and the peak or acrophase (Æ, time of maximum in the fitted cosine function, with local midnight of December 22 as Æ reference). Data were also pooled separately into three different seasons, namely rainy (July to October), winter (November to February), and summer (March to June) for further comparison. One-way analysis of variance (ANOVA), followed by the *post hoc* Duncan's multiple-range test, was applied to determine the differences in activity duration as function of season. Pearson's *r* was computed to reveal relationship between the activity duration (dependent variable) and several independent variables, such as day length, sunrise time, sunset time, morning/evening temperature, and morning/evening humidity. Stepwise multiple linear regression (MLR) analysis was carried out to identify the most significant predictor of the activity duration. To ensure that there are no missing data in different sets of time series, data belonging to the first three months and

Table 1. Mean and range of environmental variables (n = 164) at the roosting site.

Exit time			Entry time		
Environmental variable	Mean ± SE	Range	Environmental variable	Mean ± SE	Range
Sunrise time (h)	6.02 ± 0.04	5.37–6.72	Sunset time (h)	18.09 ± 0.04	17.33–18.82
Phase angle (min)*	22.55 ± 0.45	3.00–36.00	Phase angle (min)*	−30.96 ± 0.57	−54.00–−17.00
Morning temperature (°C)	21.03 ± 0.48	8.20–31.30	Evening temperature (°C)	27.99 ± 0.39	18.00–40.50
Morning humidity (%)	81.16 ± 1.04	42.00–99.00	Evening humidity (%)	60.90 ± 1.70	11.00–98.00

*Difference between sunrise time/sunset time and exit of the first bird/entry of the last bird, respectively.

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four days in July 2010 were excluded from correlation and MLR analyses. All statistical analyses, except Cosinor rhythmometry, were performed using SPSS for Windows.

Results

Emergence activity

The time of emergence of the first bird was measured as the key indicator of the onset of activity for the entire colony. Initially, emergence of birds takes place either as a single individual or as small flocks about half an hour before sunrise over the seasons (Table 1). About 3–5 min after the initial emergence, flocks were apparently noticed, which varied in size between 5 and 15 individuals. At the peak period of emergence, the flight occurred in series of separate flocks subsequently following each other at short intervals. Thereafter, the emergence of individuals slowed down, and gradually a small number of single individuals appeared intermittently till the dawn.

Entrance activity

It was difficult to monitor the onset of return flight precisely, because birds displayed both inward and outward flights periodically throughout twilight period. Approximately, half an hour before sunset, their activity above the roosting site steadily increased and they exhibited numerous circling flights at an altitude of few meters. Entry flights were characterized by appearance of few birds, which increased progressively to small flocks and attain their peak at pre-dusk. The time at which the last bird arrived to the roost was regarded as to imply the end of activity of the entire colony and was about half an hour after sunset over the seasons (Table 1).

Circannual rhythm in activity duration

A statistically significant ($p < 0.001$) circannual rhythm ($t = 12$ months) in the activity duration was validated. The peak was noticed on 22 June with a spread ranging between 19 June and 24 June (Figure 2). The circannual rhythm characteristics for activity duration are shown in the legend to Figure 2.

Effect of season on activity duration

A statistically significant effect of season on activity duration was obtained (ANOVA: $F_{2,185} = 294.65$; $p < 0.001$). Result of Duncan's multiple-range test indicated that the duration of activity was the smallest in winter and was the maximum in summer (Figure 3).

Relationship of activity duration with independent variables

Pearson's correlation analyses revealed that activity duration exhibited positive relationship with day length, sunset time, morning temperature, and evening temperature (all at $p < 0.001$) (Table 2). A negative relationship between activity duration and sunrise time or morning humidity (both at $p < 0.001$) was discerned (Table 2). However, no relationship was found between activity duration and evening humidity.

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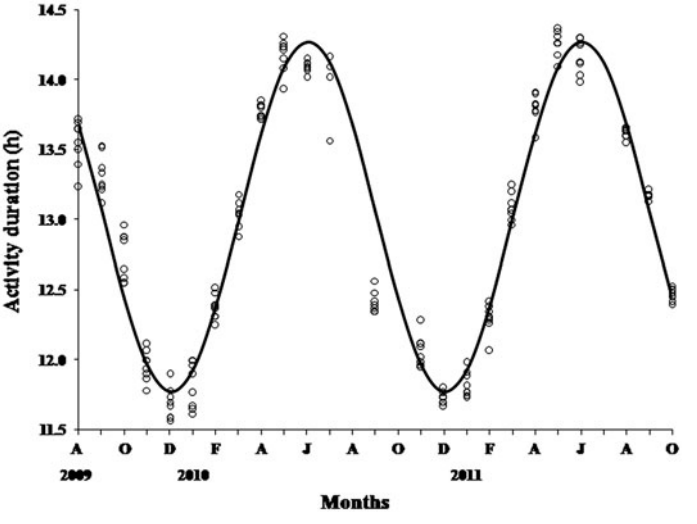

Figure 2. Circannual rhythm in activity duration of Indian cliff swallow, *H. fluvicola*. The fitted line represents the best-fit Cosine curve (t = 12-month). Rhythm parameters: Mesor = 13.01 ± 0.01; Amplitude = 1.25 (1.2, 1.3); Acrophase = June 22 (June 19, June 24). Values within the parenthesis denote 95% confidence limit.

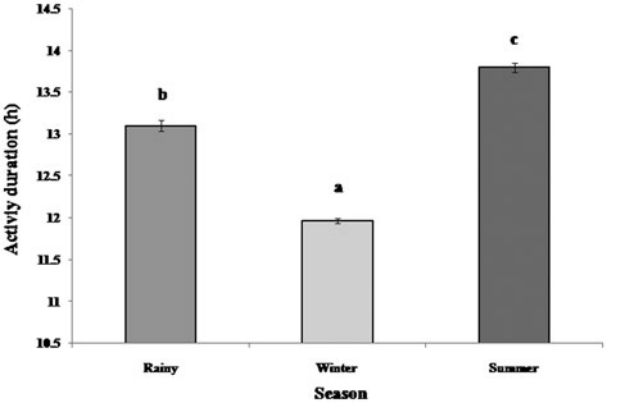

Figure 3. Seasonal variation in activity duration of the colony of Indian cliff swallow, *H. fluvicola* (ANOVA Summary: $F_{2,185} = 294.65$; $p < 0.001$). Means bearing similar alphabets do not differ from each other at $p < 0.05$, based on Duncan's multiple-range test.

Predictors of circannual rhythm in activity duration

Results of stepwise MLR analysis revealed that the day length contributed to this model to the tune of 96.0% (refer Model 1 in Table 3). In the model 2, that includes both day length and evening temperature, the overall contribution was 96.5% (Table 3).

Discussion

The peak of the circannual rhythm in activity duration was detected in the last week of June. Why do they forage for a longer period in June – the period with soaring temperature? There are several possible explanations. Firstly, June is the month that precedes

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Table 2. Summary of correlation coefficients (r) between independent variables and activity duration (dependent variable).

Independent variable	Activity duration			
	n	R^2	r	p-value
Day length	164	0.960	0.980	<0.001
Sunrise time	164	0.888	−0.942	<0.001
Sunset time	164	0.850	0.922	<0.001
Morning temperature	164	0.694	0.833	<0.001
Evening temperature	164	0.535	0.731	<0.001
Morning humidity	164	0.115	−0.340	<0.001
Evening humidity	164	0.006	−0.075	0.343

Table 3. Results from stepwise MLR analyses examining the effects of sunrise time, sunset time, day length, morning temperature, evening temperature, morning humidity, and evening humidity on activity duration (dependent variable).

Model	r	R^2	B	SE	F-ratio	df	p-value
Model 1[a]	0.980	0.960	1.000	0.016	3848.73	1,162	<0.001
Model 2[b]	0.982	0.965	1.204	0.194	2237.12	1,161	<0.001

[a]Day length.
[b]Day length, Evening temperature.
Note: SE – Standard error; df – Degrees of freedom.

the occurrence of monsoon in Chhattisgarh, when insects are abundantly available. Secondly, the birds attempt to accumulate more energy prior to the period when they are expected to construct/reconstruct nests, and to get ready for egg laying and parental care. Thirdly, it could be an expression of endogenous circannual rhythm that has been evolved to guarantee maximum reproductive fitness to the species. In this study, none of these possibilities have been examined experimentally. Nonetheless, we surmise that since the bird under investigation is insectivorous, probably it synchronizes its expected egg laying time with the onset of monsoon, when insects are plenty. Long ago, Lack (1950) was the first to propose that the birds breed at the appropriate time along the annual time scale, when there is plenty of food in its neighborhood, and the ambient temperature is optimum. It was later supported by Berthold (2001), who alleged that birds make physiological and behavioral adjustments to ensure that breeding occurs at the most appropriate period of the year. The availability of food may also act as a temporal cue, independent of the day length (Hahn et al. 2005). Normally, in tropical regions, the onset of monsoon is closely associated with the abundance of insects (Immelmann 1971). It has been observed that a predictable synchronizer for circannual rhythm may vary as function of species and in some cases as function of geographic regions (Bradshaw & Holzapfel 2007; Newton 2008; Foster & Kreitzman 2009). Furthermore, the synchronizer may also be different for different populations within the same species (Scheuerlein & Gwinner 2002; Carey 2009). In many species of birds, especially the long day breeders those inhabit high latitudes, the day length and an endogenous rhythm of photosensitivity together work as determinant of the phases of annual cycles of reproduction (Helm et al. 2009).

When duration of activity was viewed apropos of the major seasonal compartments, it was found to be the shortest in winter, the longest in summer, and was intermediate

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in rainy season. It has been established since very long that day length (photoperiod) influences seasonal timing of reproduction (Rowan 1925; Thapliyal 1978; Dawson et al. 2001). However, there are limited studies to account for interannual variation (Caro et al. 2013). The presence of interannual variation proves the point that the day length is not the only factor that defines seasonality in many physiological and behavioral variables. The phenomenon of seasonality can be defined by two important features, namely the amplitude of the seasonal variations and the accuracy with which these variations take place every year (Wingfield et al.1992; Visser et al. 2010). Most of the species have been evolved to stay in sync with the seasonal variability in order to enhance their fitness for survival (Hau 2001; Helm et al. 2009). The seasonality is a complex phenomenon. It is not the changes in day length alone; rather, alterations in several other factors; for instance, temperature, humidity, precipitation, and foraging material, in addition to day length along with the annual time scale constitute seasonality. The physiological and behavioral output in birds varies dramatically as function of season. In few species, the activity declines at the advent of hotter period (Engel et al. 1992; Janicke & Chakarov 2007), while in others the activity increases considerably (Peh 2002; Everding & Jones 2006). This species belongs to the latter type as the foraging period of its colony was longer in the summer months characterized by long day length and high temperature.

A positive linear regression curve was obtained when the activity period was plotted in relation to the day length. In day, active birds, both in nature and in captivity, where there was large fluctuation in day length, activity period becomes independent of the day length. An S-curved relationship has been reported for those species when daily activity period was plotted against photoperiod (Aschoff 1969; Aschoff et al. 1970; Daan & Aschoff 1975). However, in tropical regions, an S-curved relationship has not been reported. The best example is the present study, wherein a positive relationship between day length and activity duration has been discerned. In tropical and subtropical regions, annual variation in day length is the least (Pinet et al. 2011). Therefore, many researchers are of the opinion that probably photoperiod is of little use in the governance of seasonal rhythmicity in metabolic and reproductive behavior (Immelmann 1971). However, this hypothesis is not tenable. The present study reveals a direct positive relationship between day length and duration of activity along the longitudinal time scale. In equatorial birds, the annual changes in the timings of sunrise, sunset, and the day length act as potential *zeitgeber* for circannual rhythms (Goymann et al. 2012). It has been postulated that at lower latitudes, birds rely upon additional cues to predict their breeding, metabolic, and behavioral activities (Wingfield et al. 1992).

In many avian species, the length of daily activity has been shown to have a relationship with ambient temperature (Inouye et al. 2000; Dunn 2004; Salvante et al. 2010; Singh et al. 2012) and relative humidity (Peh 2002; Gordo 2006; Verma 2010) . In *Otis tarda* (Martínez 2000), *Brachyramphus marmoratus* (Naslund & O'Donnell 1995), and *Junco phaeonotus* (Weathers & Sullivan 1993), the length of activity decreases during winter months. However, in *Sturnus vulgaris*, the activity duration remains unaffected by the ambient temperature (Eiserer & Thompson 1989). The daily activity of Psittacids inhabiting Amazonian forest has been shown to exhibit bimodal activity; the birds remain less active during the hotter part of the day (Roth 1984). It has been shown that temperature has a link with the availability of insects, and therefore many insectivorous species modulate their activity period accordingly (Racey & Swift 1985). In the present study, the activity duration was found to be positively correlated with the morning and evening temperatures.

The relative humidity also influences both the duration and intensity of activity. Normally, high humidity has been linked with the reduced activity in *Progne subis* (Finlay 1976), *Chaetura pelagica* (Zammuto & Franks 1981), and *Corvus splendens* (Peh 2002). Results of the study corroborate with the above findings. We observed a negative correlation between the morning humidity and activity duration in this species.

Although correlation analyses, performed in the present study, explain circannual rhythm in activity duration attributed to several of the tested independent variables, it is not clear how strongly they act as predictors of the rhythm. The results that emerged from stepwise MLR analyses helped us to subtract the role of many independent variables, excluding day length and evening temperature, as predictors of the rhythmic phenomenon. The MLR models suggest that day length alone explains 96% of the total variance in activity duration, whereas only 0.5% variance was attributable to evening temperature. The findings of this study establish that the day length is the strongest predictor of circannual variation in activity duration. Further, the findings of this study will augment our knowledge on the biological consequences of climatic transformation on the activity duration of the bird. Comparable studies under natural conditions might be very helpful to explore the effect of environmental cues on other intriguing behavioral decisions made by this and other avian species.

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Temporal Pattern in Roosting Behavior of the House Swift, *Apus affinis* with Reference to Environmental Factors – a Longitudinal Study

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Abstract. There is complete lack of information on roosting behavior of the house swift, *Apus affinis* native to Chhattisgarh. Therefore, we studied the roosting behavior (exit and entry patterns from/to its roosting site) of the house swift. Attempts were also made to ascertain the modulatory role of environmental factors. We monitored the exit and entry timings of the house swift in the roosting site located on the busiest commercial area of Raipur city, India, for eight consecutive days, every month over a period of two years. The exit and entry timings showed positive relationship with the sunrise and sunset, respectively. The house swift exited later and entered earlier in winter as compared with summer and monsoon seasons. A significant negative correlation between exit time and morning ambient temperature, and positive correlation between entry time and evening temperature was witnessed. Unlike exit time, the entry time was negatively associated with the humidity. The entry time was also associated with light intensity. In summary, we conclude that various environmental factors, *viz*., sunrise time, sunset time, temperature, humidity and light intensity are likely to produce statistically significant effect on roosting behavior of the house swift. This study is, however, inadequate to answer the following questions: (1) how the first bird of the colony determines that it is the time to fly out? (2) Is it the same individual that flies out first daily? Nonetheless, the findings of the present study may have important bearings on the safety of civil and military flights.

Keywords: Exit & entry timings, longitudinal study phase angle, seasonal variation, sunrise, sunset

Introduction

A number of research papers have been published describing the roosting behavior of avian species, such as migratory common swifts (*Apus apus*) and European alpine swifts (*A. melba*) (Koskimies, 1950; Lack and Lack, 1952; Church, 1956; Lack, 1956), starlings (*Sturnus vulgaris*) (Davis, 1955; Davis and Lussenhop, 1970), house swift (*A. affinis*) (Razack and Naik, 1965), herring gull (*Larus argentatus*) (Schreiber, 1967), Canada geese (*Branta canadensis*) (Raveling *et al.,* 1972), black-capped chickadees (*Parus atricapillus*) (Kessel, 1976), rooks (*Corvus frugilegus*) (Swingland, 1976), domestic fowl (*Gallus gallus domesticus*) (Wood-Gush *et al.*, 1978; Kent *et al*., 1996), wintering black ducks (*Anas rubripes*) (Brodsky and Weatherhead, 1984), magpies (*Pica pica*) (Reebs, 1986, 1987), common myna (*Acridotheres tristis*) and white-vented myna (*A. javanicus*) (Kang and Yeo, 1993; Gupta and Goel, 1994; Jayson and Mathew, 1995), house crow (*C. splendens*) (Peh, 2002) and common ravens (*C. corax*) (Janicke and Chakarov, 2007). In about fifty percent of these papers, roosting behavior has been studied in relation to geophysical variables. However, there is not even a single report on temporal pattern of roosting behavior of house swift along longitudinal time scale.

House swift is a small sized, quick flier, insectivorous and colonial nester bird. It is renowned for its speediness in the flight. It belongs to the Order - Apodiformes and Family – Apodidae. Swifts species are dispersed all over the planet (Lee *et al*., 1996). The humming birds are considered as the predecessor for the swifts (Sibley and Ahlquist, 1990; Bleiweiss *et al*., 1994; Lee *et al*., 1996; Johansson *et al*., 2001, Livezey and Zusi, 2001; Mayr, 2002, 2003; Thomassen *et al*., 2005; Ksepka *et al*., 2013). House swift lives near the human habitation; sometimes in noisy or deafening downtown (Wotton *et al*., 2002). The major activity time of swifts is spent on aerial foraging (Church, 1956). Swift birds are also known as Layang Layang (which means kite, Malay name); because in most part of the day, they are airborne. Swifts cannot sit down wherever due to very skinny, small feet and some may even have difficulty taking flight again if they land on the ground (http://www.naturia.per.sg/buloh/birds/Apus_nipalensis.htm). The North American vaux's swift (*Chaetura vauxi*) during the reproductive phase build nests in large-sized tree trunks (Baldwin and Zaczkowski, 1963; Bull, 1991; Bull and Cooper, 1991); however, similar observations are not available for house swift.

The diet of swifts comprises of different groups of arthropods, such as small flying insects, flies, mosquitoes, midges, flying ants, termites, dragonflies, grasshoppers, spiders and moths (Glick, 1939; Lee *et al*., 1996). Normally the birds feed on

aerial bugs, while on flight, up to 300 m over the ground (Berland, 1935; Glick, 1939). The foraging region of airborne insectivorous birds extends over large area at the maximum altitude (Norberg, 1986; Warrick, 1998). The density of the aerial insectivorous birds is directly proportional to the amount of the airborne insects (Chapman *et al.*, 2003). There is evidence to presume that the extinction of many aerial forager birds is due to non-availability of insects up in the air, urbanization, extensive use of pesticides and insecticides in fields (Brown, 1985; Bull, 1991; Bull and Hohmann, 1993).

Knowledge of the roosting behavior of the house swift is necessary in order to develop a long-term conservation strategy for the species. The rationale of our study was to explore temporal (daily and seasonal) variations in roosting behavior (exit and entry timings) of the house swift. In addition, we made an attempt to ascertain the relationship of various local geophysical factors, such as sunrise time, sunset time, temperature, humidity and light intensity with the exit and entry timings of the birds. We performed longitudinal study spread over a consecutive span of 24 months.

Materials and methods

Study site

We located a large colony (Figure 1a) of the house swift (*A. affinis*) (Figure 1b) in one of the old buildings in the congested noisy downtown of *Malviya Road* (climate - tropical; location 21.25˚ N; 81.63˚ E), Raipur, Chhattisgarh, India. We chose the location as the study site. The birds of the colony construct their shallow saucer or cup-shaped nests on the roof approximately 4.7 meter above the ground level. A cluster of nests looks like a big untidy mass and consists of more than a hundred individual nests each with independent passage for exit/entry.

Data collection

The study was conducted over a longitudinal time scale consisting of 24 consecutive months with effect from August 2009. In each month, we recorded the timings of exit of the first bird from the roost and entry of the last bird to the roost over a period of eight consecutive days in two sessions (morning and evening) each day. Thus, the entire study consisted of 192 days and 384 sessions. In the morning, we reached the study site an hour before and in the evening we stayed till an hour after the local sunrise and sunset timings, respectively. The sunrise and sunset timings were obtained from the meteorological department of Raipur. We recorded the temperature and humidity (using Thermo-hygrometer, Pacer®, TH-402), and light intensity (using LX-101-Lux Meter, Davis Inotek Instruments, USA) during the entire period of the study.

(a) (b)

Figure 1. Clusters of the nests of house swift (*A. affinis*) at the study site (a). Picture of a solitary house swift, *A. affinis* (b)

Statistical analysis

We stored the data in the form of a database in the MS Excel worksheet. We computed phase angles of the exit time of the first bird and entry time of the last bird with reference to sunrise and sunset timings, respectively. Correlation coefficients were computed between pairs of variables. We also identified three seasons, namely monsoon (July to October), winter (November to February) and summer (March to June) to determine the effect of season on the roosting behavior. The effect of the factor 'season' was validated using one-way ANOVA followed by Duncan's multiple-range test. Data analyses were carried out with the help of SPSS (version 10) and COSTAT Software (CoHort Software; ver. 4.02, ©).

Results

Exit & entry timings and corresponding phase angle with reference to sunrise and sunset

Figures 2 and 3 demonstrate month wise exit time of the first bird from the roost and entry time of the last bird to the roost with reference to sunrise and sunset time, respectively, along a longitudinal time scale of 24 months. It is evident that birds always exited from their nest before sunrise and entered to the roost after sunset. In an average, the birds exited from their roosts about half an hour before the sunrise time, irrespective of season and month of the year (Table 1). The last bird entered to its roost about 20 minutes after the sunset time. The negative evening phase angle complements the preceding statement in that the entry time was always after the sunset, irrespective of season and month of the year (Table 1).

Table 1. Summary of exit and entry timings of the first and the last bird, respectively, from roosting colony of the house swift, *A. affinis* and corresponding phase angle (ψ) differences with reference to sunrise and sunset timings

Season	Sunrise (h)	Exit (h)	Sunset (h)	Entry (h)	Morning ψ (min)	Evening ψ (min)
Monsoon	5.76 ± 0.02	5.28 ± 0.02	18.28 ± 0.05	18.63 ± 0.04	28.53 ± 0.97	-21.98 ± 1.52
Winter	6.51 ± 0.02	5.97 ± 0.04	17.62 ± 0.03	18.07 ± 0.03	29.37 ± 1.39	-22.99 ± 1.02
Summer	5.70 ± 0.04	5.27 ± 0.06	18.48 ± 0.03	18.85 ± 0.04	25.67 ± 1.43	-21.98 ± 0.93
Annual spread	0.81	0.70	0.86	0.78	3.70	1.01

ΨPhase angle

Further, the exit time, but not its corresponding phase angle, showed a statistically significant positive relationship with sunrise (Table 2). Both the entry time and corresponding evening phase angle showed statistically significant positive relationship with the sunset time (Table 2).

Exit & entry timings and corresponding phase angle with reference to ambient temperature

Exit time was significantly negatively correlated with the morning temperature, whereas entry time was positively associated with evening temperature. Further, both the corresponding phase angles exhibited insignificant relationship with the ambient temperature (Table 2).

Exit & entry timings and corresponding phase angle differences with reference to relative humidity

Exit time was positively and entry time was negatively correlated with the corresponding morning and evening humidity, respectively. Like ambient temperature, the morning and evening phase angles showed insignificant association with the respective values of humidity (Table 2).

Exit & entry timings and corresponding phase angle with reference to light intensity

Only entry time revealed a significant positive association with the evening light intensity, whereas exit time and both the corresponding phase angles showed insignificant correlation with light intensity (Table 2).

Effects of season

Results of ANOVA indicated that the factor 'season' produced a significant ($P < 0.001$) effect on exit time. The birds exited later during winter in terms of local time (exit time: 5.97 ± 0.04 hr; phase angle: 29.37 ± 1.39 min), but earlier during monsoon (exit time: 5.28 ± 0.024 hr; phase angle: 28.53 ± 0.97 min) and summer seasons (exit time: 5.27 ± 0.06 hr; phase angle: 25.67 ± 1.43 min) with respect to sunrise time (Table 1; Figure 4a).

Likewise, factor 'season' produced a statistically significant ($P < 0.001$) effect on entry time. The bird entered in the roost significantly earlier during the winter season (entry time: 18.07 ± 0.03 hr; phase angle: -22.99 ± 1.02 min), followed by monsoon (entry time: 18.63 ± 0.04 hr; phase angle: -21.98 ± 1.52 min) and subsequently during the summer season (entry time: 18.85 ± 0.04 hr; phase angle: -21.98 ± 0.93 min) with respect to sunset time (Table 1; Figure 4b).

However, the factor 'season' did not produce any significant effect on the corresponding morning and evening phase angles (Figure 5).

Table 2. Summary of correlation coefficients (r) between pairs of dependent and independent variables

Independent variables	Dependent variables	n	r	P value
Sunrise time (h)	Exit time (h)	194	0.926	**0.001**
	Morning phase angle, ψ (min)	194	-0.036	0.617
Sunset time (h)	Entry time (h)	194	0.923	**0.001**
	Evening phase angle, ψ (min)	194	0.246	**0.001**
Morning temperature (°C)	Exit time (h)	194	-0.676	**0.001**
	Morning phase angle, ψ (min)	194	-0.057	0.426
Evening temperature (°C)	Entry time (h)	194	0.613	**0.001**
	Evening phase angle, ψ (min)	194	0.129	0.072
Morning humidity (mmHg)	Exit time (h)	170	0.398	**0.001**
	Morning phase angle, ψ (min)	170	0.020	0.798
Evening humidity (mmHg)	Entry time (h)	170	-0.393	**0.001**
	Evening phase angle, ψ (min)	170	-0.045	0.556
Morning light intensity (lux)	Exit time (h)	170	0.073	0.342
	Morning phase angle, ψ (min)	170	0.136	0.078
Evening light intensity (lux)	Entry time (h)	170	0.700	**0.001**
	Evening phase angle, ψ (min)	170	-0.036	0.640

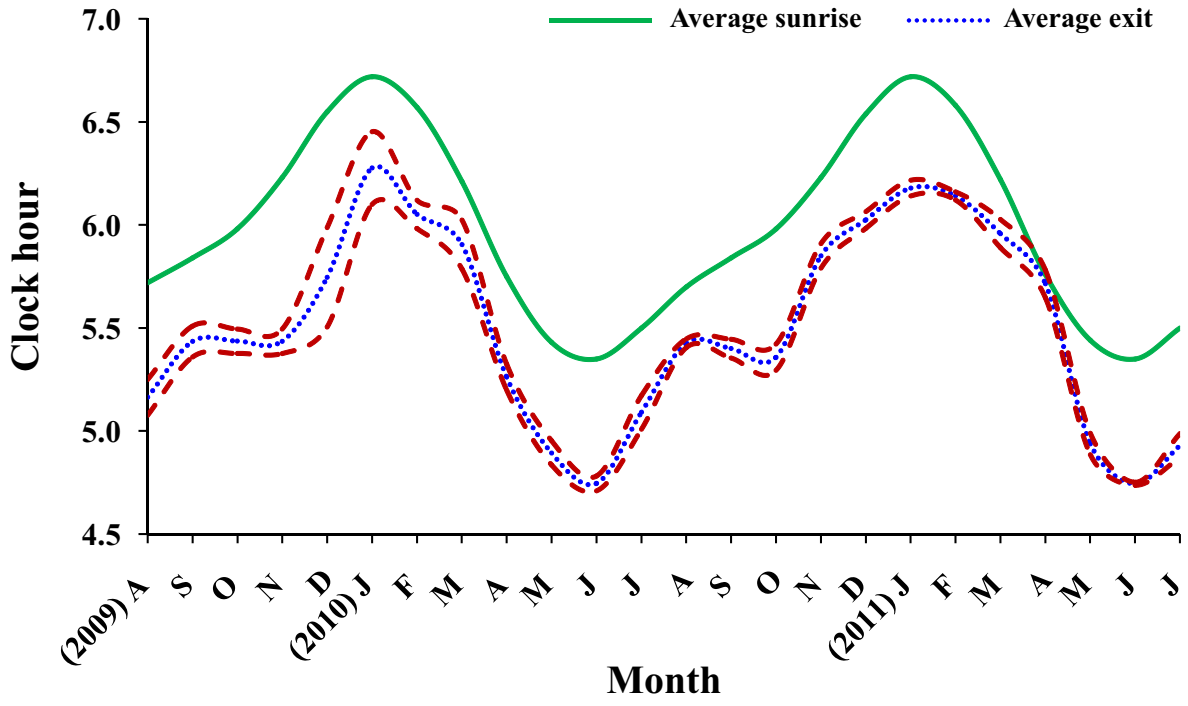

Figure 2. Sunrise time and exit time of the first bird from the nest (from August 2009 to July 2011). The dotted lines around the average exit time indicate 95% confidence limit.

Discussion

Roosting behavior (exit and entry timings) with reference to environmental cues has been studied in a number of avian species (see introduction section). We report here, for the first time, the roosting behavior of house swift (*A. affinis*). The species under study is native to Chhattisgarh, India.

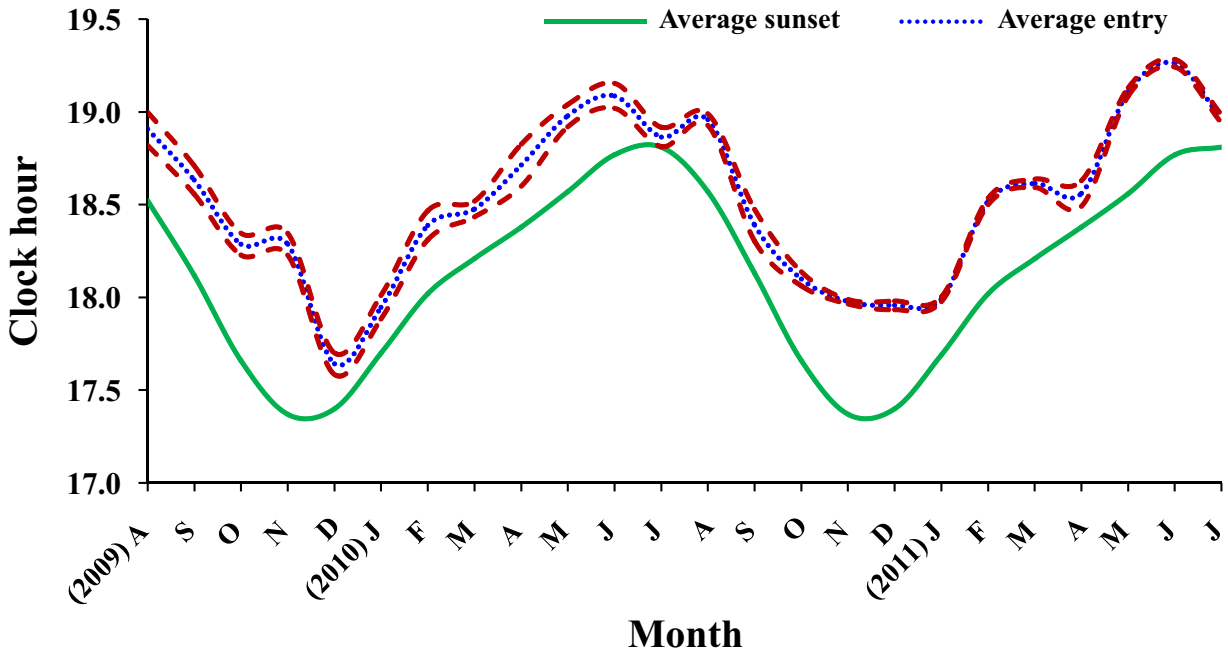

Figure 3. Sunset time and entry time of the last bird to the nest (from August 2009 to July 2011). The dotted lines around the average entry time indicate 95% confidence limit.

Sunrise and sunset time

The present study reveals that sunset time is a dominant time cue for roosting behavior, as the annual spread of evening phase angles appears to be the least (1.01 min) and more precise. It has been argued that the sunrise and sunset timings are the leader factors (Michael and Chao, 1973; Zammuto and Franks, 1981) followed by the other environmental factors, such as temperature, wind velocity, and cloud cover. Different roosting activities, such as wake up, exit, and entry timings show diurnal and seasonal variations apropos timings of sunrise and sunset (Mahabal and Vaidya, 1989). In the present investigation, house swift (*A. affinis*) exited their roost about half an hour before sunrise time and entered in to the roost around 20 minutes after sunset time. Our findings draw support from earlier studies conducted on other swift species (Michael and Chao, 1973; Zammuto and Franks, 1981; Ryan and Collins, 2003). In contrast, house swifts (*A. affinis*) exhibits a wider phase angle (range: 1.25 to 3.25 hr) between sunrise and exit time (Razack and Naik, 1965). According to Arn-Willi (1960) in case of the Alpine swift (*A. melba*), Peh (2002) in case of the house crow (*C. splendens*) and Reebs (1987) in case of black-billed magpies (*P. pica*) the timings of exit and entry are diametrically opposite. They exit from their roost after the dawn and return considerably before the dusk. On the other hand, roosting time in higher altitudes is comparatively earlier as reported in the common swift (*A. apus)* found in Germany and Finland and has been attributed to greater twilight span in that region (Scheer, 1949). In domestic fowl (*G. gallus domesticus*) the roosting timings have been strongly correlated with the sunrise and sunset timings (Wood-Gush *et al*., 1978). The entry time has also been shown to have a significantly positive correlation with the sunset time in the house crow (*C. splendens*) (Peh, 2002), common myna (*A. tristis*) and white-vented myna (*A. javanicus*) (Kang and Yeo, 1993; Gupta and Goel, 1994; Jayson and Mathew, 1995).

Temperature

In the present study, the roosting time of house swift (*A. affinis*) was found to be significantly influenced by the ambient morning and evening temperature. Comparable findings have been reported in common swift (*A. apus*) (Koskimies, 1950), house swift (*A. affinis*) (Razack and Naik, 1965), chimney swift (*Chaetura pelagica*) (Zammuto and Franks, 1981), white-throated swift (*Aeronautess axatali*) (Ryan and Collins, 2003), Canada geese (*B. canadensis*) (Raveling *et al*., 1972), black-capped chickadees (*P. atricapillus*) (Kessel, 1976), black ducks (*A. rubripes*) (Brodsky and Weatherhead, 1985), starlings (*S. vulgaris*) (Jumber, 1956), house crow (*C. splendens*) (Peh, 2002) and magpies (*P. pica*) (Reebs, 1986). At lower temperature, the house swift (*A. affinis*) exited later and entered to the roost earlier. It has been demonstrated that food accessibility decreases in lower temperature; therefore, birds spend most of the time in the nest and reduce their activity (Reebs, 1986). The availability of arthropods up in the air has been shown to be influenced by the irregularity in temperature (Holm and Edney, 1973; Romoser and Stoffolano, 1994). The favorable range of temperature from 22°C to 38°C and up to 60 m above the ground for exit has been reported for common swifts (*A. apus*) (Glick, 1939, 1957; Koskimies, 1950). In contrast, there are many avian species, wherein their roosting behavior remains independent of factors, like temperature, rain and blustery weather (Janicke and Chakarov, 2007 for Common ravens, *C. corax*; Schreiber, 1967 for herring Gull, *L. argentatus*). Furthermore, while entry time of house crow (*C. splendens*) was positively associated with the temperature (Peh, 2002); the entry, but not exit time, of the magpies (*P. pica*) was significantly influenced by the temperature (Reebs, 1986).

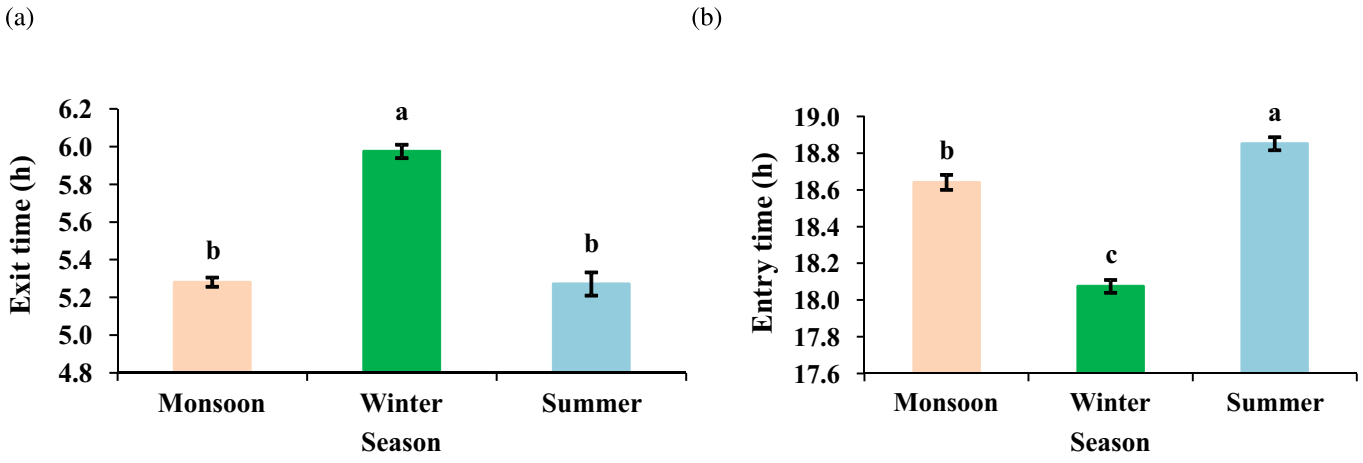

Figure 4. Seasonal variation in exit (a) and entry (b) timings of house swift (*A. affinis*) during the study period. ANOVA summary: exit time: $F_{2,191} = 87.16$, $P < 0.001$; entry time: $F_{2,191} = 117.02$, $P < 0.001$. Means bearing the same letter are not significantly different from each other at $P < 0.05$ (based on Duncan's multiple-range test).

Humidity

The exit and entry timings of the house swift (*A. affinis*) was found to be influenced by humidity. In swifts both visual (rain and clouds) and tangible (wet condition) signals have been shown to influence both roosting and foraging behaviors (Ryan and Collins, 2003). The entry time has been shown to be negatively correlated with humidity in house crow (*C. splendens*) (Peh, 2002) and chimney swifts (*C. pelagica*) (Zammuto and Franks, 1981).

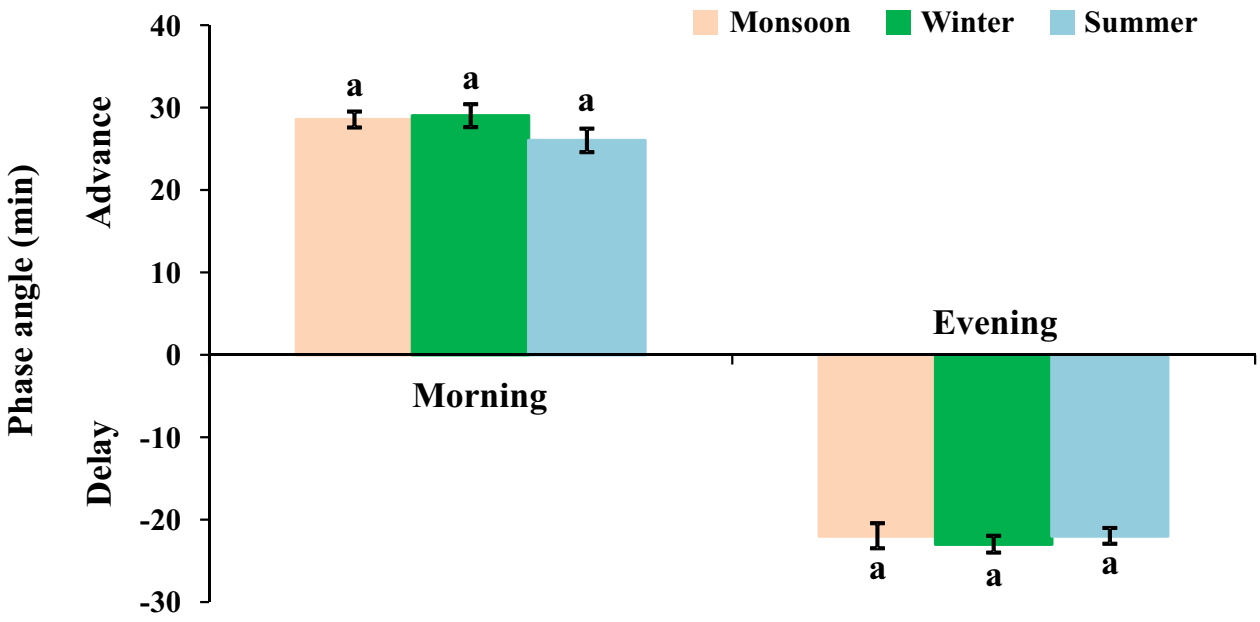

Figure 5. Seasonal variation in morning and evening phase angle of exit and entry timings of the first and the last bird, respectively. ANOVA summary: Morning phase angle: $F_{2,191} = 2.26$; $P = 0.11$; Evening phase angle: $F_{2,191} = 0.40$; $P = 0.79$). Means bearing the same letter are not significantly different from each other at $P < 0.05$ (based on Duncan's multiple-range test).

Light intensity

In the present study, the entry time was found to be significantly correlated with the light intensity. Similar relationship has been reported earlier in other species of the swifts, like common swifts (*A. apus*), Alpine swifts (*A. melba*), house swifts (*A. affinis*) and chimney swifts (*C. pelagica*) (Koskimies, 1950; Church, 1956; Razack and Naik, 1965; Michael and Chao, 1973; Zammuto and Franks, 1981). In addition, such relationship has also been observed in other bird species, such as starlings (*S. vulgaris*) (Davis, 1955; Jumber, 1956; Davis and Lussenhop, 1970), rooks (*C. frugilegus*) (Swingland, 1976), magpies (*P. pica*) (Reebs, 1986) and domestic fowl (*G. gallus domesticus*) (Kent *et al*., 1996). The broad spectrum of light intensity has been reported to be required for the exit than entry in chimney swifts (*C. pelagica*) (Zammuto and Franks, 1981). Michael and Chao (1973) reported that chimney swifts (*C. pelagica*), entered to the roost at higher light intensities during colder months, whereas they entered at lower light intensities during warmer months. Contradictory findings have been reported in white-throated swifts (*A. saxatalis*) (Ryan and Collins, 2003).

According to Ryan and Collins (2003) temperature is the foremost *zeitgeber* for the insect abundance in the air than light intensity, which affects the roosting behavior of white-throated swift (*A. axatali*), whereas the flight and foraging are accurately predicted by light intensity. The prominent influencing factors for the roosting time of herring gull (*L. argentatus*) have been reported to be light intensity, cloud cover and daylight span as compared to temperature and wind characteristics (Schreiber, 1967). The herring gull (*L. argentatus*) entered to their roost prior to night (Schreiber, 1967). Cloud cover influenced indirectly the roosting time of house crow (*C. splendens*) on account of decreased light intensity; however, entry time was not significantly associated with light intensity (Peh, 2002). At 1-5 lux, most of the magpies (*P. pica*) exited from their roost; it implies that the minimum range of light intensity is required for safe flight, whereas the entry to the roost takes place at wide range of light intensity between 24.2-1174.2 lux. These results suggest that higher and variable range of light intensities is required for the entry as compared for the exit (Reebs, 1986).

Season

In the present study, we observed a statistically significant effect of season on exit and entry pattern/ roosting behavior of the house swift (*A. affinis*). Similar observations have been demonstrated for herring gull (*L. argentatus*) (Schreiber, 1967), the chimney swifts (*C. pelagica*) (Zammuto and Franks, 1981) and white-throated swifts (*A. saxatalis*) (Ryan and Collins, 2003).

In an earlier study, it has been demonstrated that the exit of the white-throated swifts (*A. saxatalis*) from their roost is influenced by daily variation in the climatic conditions as compared to seasonal changes; whereas entry fluctuates more predictably by season than climatic conditions (Ryan and Collins, 2003). We found that the house swift (*A. affinis*) exited later during winter as compared to monsoon and summer seasons, and entered to the roost significantly earlier during the winter season than monsoon and summer season. This phenomenon could be attributed to temperature-linked dispersal of arthropods that in turn affects foraging length directly and the activity period and roosting behavior indirectly (Koskimies, 1950). The chimney swifts (*C. pelagica*) (Zammuto and Franks, 1981; Ryan and Collins, 2003), house crow (*C. splendens*) (Peh, 2002), rooks (*C. frugilegus*) (Swingland, 1976; Hubalek, 1978), common crow (*C. brachyrhynchos*) (Haase, 1963), Canada geese (*B. canadensis*) (Raveling *et al*., 1972), captive great tits (*Parus major*) (Dunnett and Hinde, 1953) and starlings (*S. vulgaris*) (Davis and Lussenhop, 1970) exited later and returned earlier in the cloudy and rainy season compared to winter and clear days. In the clear day, common ravens (*C. corax*) entered to their roost later in the evening (Janicke and Chakarov, 2007). According to Reebs (1986), the magpies (*P. pica*) have been shown to exit from their roost later and enter earlier on the cloudy days, cold days, and long days than during the shorter days (winter) and mild days. The parallel results have been reported for wintering black ducks (*A. rubripes*) (Brodsky and Weatherhead, 1984) and black-capped chickadees (*P. atricapillus*) (Kessel, 1976). In winter, white-throated swifts (*A. saxatalis*) entered their roost close to or subsequent to sunset, but in spring and summer prior to sunset (Ryan and Collins, 2003). In contrast, the house swift (*A. affinis*) exited from the roost earlier and returned to the roost later; and the longest phase angle was found during the longest activity period in summer. In several species, foggy weather and rainfall have been shown to influence the late exit from the roosts (Koskimies, 1950; Lack and Lack, 1952; Razack and Naik, 1965; Michael and Chao, 1973; Zammuto and Franks, 1981). Early return to the roost in several species of swifts has been attributed to unfavorable conditions, such as smog and rain (Lack and Lack, 1952; Church, 1956; Razack and Naik, 1965; Zammuto and Franks, 1981). Following initiation of cloudburst, common swift (*A. apus*) and chimney swifts (*C. pelagica*) have been observed to return to the roost immediately (Lack and Lack, 1952; Zammuto and Franks, 1981).

Conclusion

We conclude that various environmental factors, *viz*., sunrise time, sunset time, temperature, humidity and light intensity are likely to produce statistically significant effect on exit and entry timings or roosting behavior of the house swift (*A. affinis*). The sunset appears to be one of the most dominant time cues for the activity period in house swift (*A. affinis*), especially because entry time was found to be significantly associated with all the independent variables considered in the present study. In addition, the factor 'season' has significant modulatory role on roosting behavior. The present study is, however, inadequate to answer the following questions: (1) how the first bird of the colony determines that it is the time to fly out? (2) Is it the same individual that flies out first daily? The findings of the present study may have important bearings on the safety of civil and military flights.

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Effect of Coke Oven Effluent on Serum Gamma Globulin and A/G Ratio in *Clarias batrachus*

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Abstract. The impact of both short term and long term coke oven effluent released from the Bhilai Steel Plant (BSP) has been studied on *Clarias batrachus* Linn. (1758) with particular reference to A/G ratio and serum gamma globulin. The model animal was given short term exposure in 30% of coke oven effluent for five days and long term exposure was given for six weeks in 0.5% effluent. The A/G ratio and gamma globulin were determined in serum by standard methods. Experimental findings depicted augmentation in A/G ratio and gamma globulin as a result of short term exposure, whereas long term exposure resulted in significantly high A/G ratio. Significant effect of time and group was confirmed in the gamma globulin concentration. This study reveals negative impact of coke oven effluent, released from the BSP, on local fauna.

Keywords: Coke oven effluent, Bhilai Steel Plant, *Clarias batrachus*, A/G ratio, gamma globulin, phenol

Introduction

Massive deteriorations of water qualities have ensued with elapsing time due to the release of effluents from domestic, industrial and agricultural sources. In order to keep a check on the pollution, a large number of toxicants are being identified and their recommended values documented by the United States Environmental Protection Agency (2009).

The Bhilai Steel Plant is an integrated steel plant situated 30 kilometers (west) of Raipur, the capital of the state of Chhattisgarh, India [Latitude:21°13'N; Longitude:81°23'E]. The major marketable product is good quality steel produced along with important by products, such as, Coal tar, Naphthalene and Benzol. The metallurgy of iron from its ore requires coke as a fuel and reducing agent in the blast furnace. This coke is converted from Bituminous coal in the coke ovens. The effluent generated in the coke ovens has a strong phenolic odor and contains a high amount of Phenol ranging between 0.378 mg/l to 12.5 mg/l besides the presence of other toxic substances (Guha, 1990; Sinha, 1999; Bakde and Poddar, 2011; Mishra and Poddar, 2011, 2013a, b). Similarly, high concentrations (11.06 mg/l) of Phenols were also reported near industrial effluent channel outlets (Buikema *et al.*, 1979) and in effluents generated from refineries (Otokunefor and Obiukwu, 2005).

Phenol and phenolic compounds are stressful environmental factors presenting a threat to natural environment and human health due to their lipophilic properties (Hori *et al.*, 2006). They induce genotoxic (Jagetia and Aruna, 1997), carcinogenic (Tsutsui *et al.*, 1997) and immunotoxic effects (Taysse *et al.*, 1995) on fish health, besides reduction in fish weight and fertility (Saha *et al.*, 1999). Polycyclic aromatic hydrocarbons and polychlorinated biphenyls are endocrine disrupters (Kashiwada *et al.*, 2002; Pait and Nelson, 2003; Barse *et al.*, 2006; Martin-Skilton *et al.*, 2006; Tollefsen, 2007) and adversely affect fish metabolism (Gupta *et al.*, 1983; Abdel-Hameid, 1994). Most importantly, Phenol has been listed in the National Recommended Water Quality Criteria (NRWQC) as a priority pollutant with an organoleptic effect criterion of 300µg/l (USEPA, 2009). Hence, it has a unique quality of tainting the taste of fish if present in marine environments at 0.1-1.0 ppm (Kirk and Othmer, 1982; Neff, 2002). Thus, the importance of taking into consideration Phenol intoxication in natural aquatic habitats becomes evident.

Acute exposure to environmental changes results in innate immune response whereas the chronic exposure results in adaptive immune response (Bowden, 2008). Stress leads to alteration in the immunoglobulin level (Varsamous *et al.*, 2006). A/G ratio is the index to find out the relative changes in the composition of serum or plasma (Jacobs *et al.*, 1990). This work is an attempt to ascertain the adverse effects of coke oven effluent of Bhilai Steel Plant on the immune response in the fish, *Clarias batrachus.*

Material and methods

Collection and acclimatization of experimental model

Healthy and apparently disease free catfish (*Clarias batrachus*) within the range of 230 ± 20 g weight and 30 ± 2 cm length were collected from local fish markets at Raipur, Chhattisgarh and transported to the laboratory alive. They were then acclimatized in dechlorinated tap water for a period of 15 days at 28 ± 1 °C in a glass aquarium of size (4×3 ft) along with aeration throughout. Fish food was provided twice every day.

Collection and analysis of coke oven effluent

The Coke oven effluent was collected from its source near *Purena nala*, Bhilai-3 and brought to the laboratory on the same day. The physico-chemical characteristics, viz., pH, dissolved oxygen (DO), free CO_2, alkalinity, chloride and Phenol were measured on the same day using standard methods for the analysis of natural and treated waste water described by the American Public Health Association (1975).

Experimental design

The experimental design comprised of two types of exposures of the coke oven effluent given to the model fish (*Clarias batrachus*).

Short term exposure

On the basis of LC50 of the effluent according to Mishra and Poddar (2013a) two sublethal concentrations were prepared, viz., (A) 30% effluent containing 2.04 mg/l phenol and (B) 0.5% effluent with 0.035 mg/l phenol after dilution with dechlorinated tap water. Six live and healthy previously acclimatized *Clarias batrachus* of same size range and weight were divided into two groups consisting of three fish each. One of the groups was exposed to dechlorinated tap water which acted as control and the other was exposed to 30% coke oven effluent for 6 days in 20 liters glass aquaria. Blood samples were collected from fishes of each group by severing the caudal peduncle on the sixth day and serum was isolated for estimations of A/G Ratio (Reinhold, 1953) and Gamma globulin (Friedman, 1958). The assays were carried out on the same day in triplicates.

Table 1. Physicochemical parameters of the coke oven effluent *vis-à-vis* National Recommended Water Quality Criteria (USEPA, 2009).

Parameter	Values before exposure		NRWQC (USEPA, 2009)
	Short term	Long term	
pH	8.02	8.15	5 to 9
DO (mg/l)	6.29	6.25	-
free CO_2 (mg/l)	4.4	112.2	-
Chloride (mg/l)	69.58	58.22	860 (CMC);230 (CCC)
Alkalinity (mg/l)	0.2	0.3	2 (CCC)
Phenol (mg/l)	6.8	7.1	10*; 0.3**

*priority pollutant list; **OEC = Organoleptic Effect Criterion (USEPA, Goldbook, 1986); CMC = Criterion Maximum Concentration; CCC = Criterion Continuous Concentration

Long term exposure

Twenty-four live and healthy, previously acclimatized *Clarias batrachus* of same size range and weight were divided into two groups consisting of twelve fishes each. One of the groups was exposed to dechlorinated tap water acting as control and other to 0.5% effluent for six weeks. Blood was collected from two fishes belonging to each group at weekly intervals and serum A/G Ratio (Reinhold, 1953) and Gamma globulin (Friedman, 1958) were measured on the same day in triplicates.

Statistical analysis: Data collected were analyzed using MS Excel ToolPak (version, 2007) and COSTAT (CoHort software; version 4.02, ©).

Results

National Recommended Water Quality Criteria for Organoleptic effects (OEC) (USEPA, 2009) and the results of analysis of the physicochemical parameters of the coke oven effluent channel of Bhilai Steel Plant *vis-à-vis* NRWQC (USEPA, 2009)

are depicted in Table 1. The levels of Phenol obtained before short (6.8 mg/l) and long term exposures (7.1 mg/l) were observed to be far above the NRWQC according to USEPA (2009).

Short term exposure

A percent augmentation of 104.19 was observed in serum A/G ratio of test samples exposed to 30 % coke oven effluent for 6 days with mean values being 6.34 in test as compared from control (3.10). A percent augmentation of 9.12 was observed in serum gamma globulin of test samples being 5.62mg/ml as compared from control (5.15 mg/ml). From the results of one-way ANOVA a significant effect of factor group (Control and Test) was validated in AG Ratio (Table 2(a)) and gamma globulin (Table 2(b)) on short-term exposure (six days in 30% effluent). The mean AG ratio was found to be significantly higher in 'Test group' as compared to 'Control group' (Figure 1).

Long term exposure

Results of long term exposure to the 0.5% of coke oven effluent resulted in a very high percent hike in A/G ratio (Figure 2) of test samples as compared from control was observed in the first week (109.54) which declined to 98.09, 63.3, 49.3, 64.53 and 62.6 percent hikes in the following weeks. On the contrary, percent hike in serum gamma globulin (Figure 3) increased gradually from 2.92 in week 1 to 3.11, 4.66, 4.47, 5.06 and 4.84 in the following weeks. From two-way ANOVA the effect of factor group was also found to be significant on long term exposure of the fish *Clarias batrachus*. The 'Test group' has high A/G Ratio than the counterpart 'Control group'.The effect of factor time was found to be insignificant in mean A/G ratio (Table 2(c)).

Significant effect of factor time and group was validated in Gamma globulin concentration of *Clarias batrachus* (Table 2 (d)). From Duncan's Multiple Range Test the concentration of Gamma globulin was found to be the highest on the sixth week than the first and the second week (Table 2 (e)). The concentration of Gamma globulin was significantly higher in 'Test' than 'Control' (Figure 3). The interaction effect of factors time and group was also significant which means that both factors concentration and duration are associated in Gamma globulin concentration in *Clarias batrachus* (Table 2 (d)).

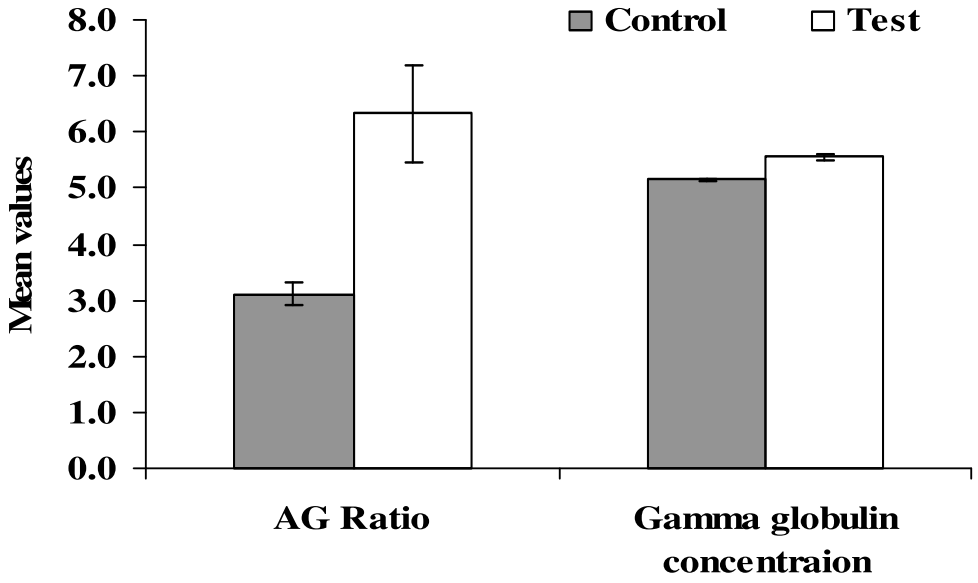
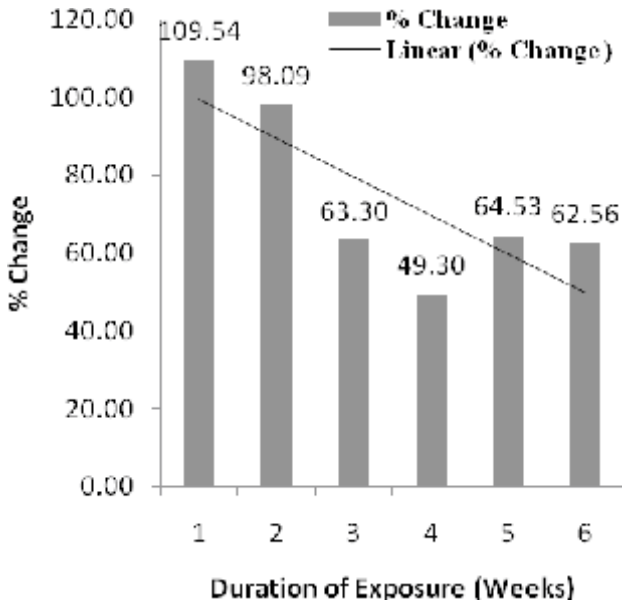


Figure 1. Mean values for AG Ratio and Serum Gamma Globulin concentration in control and test groups in response to short-term exposure to coke oven effluent of Bhilai Steel Plant.

Discussion

Increased industrial disposal of effluents to the water bodies introduces large number of toxicants to the environment whose concentration increases with time and directly or indirectly affects the health of organisms. Phenol is also one of the harmful toxicant which has hazardous impact on the health of organism. The toxicity symptoms of phenol are anemia, anorexia, in coordination, weakness and dyspepsia. Besides, centrilobular necrosis and hemorrhage of the liver have been found at post mortem (Clarke and Clarke, 1979). A study on African catfish shows that they are more tolerant to Phenol toxicity as the LC_{50} is 35mg/l which however produces deleterious effect on its various organs (Ibrahim, 2012) Also exposure to Phenol leads to impaired specific immune response and reduced disease resistance (Hamid *et al.*, 2007).



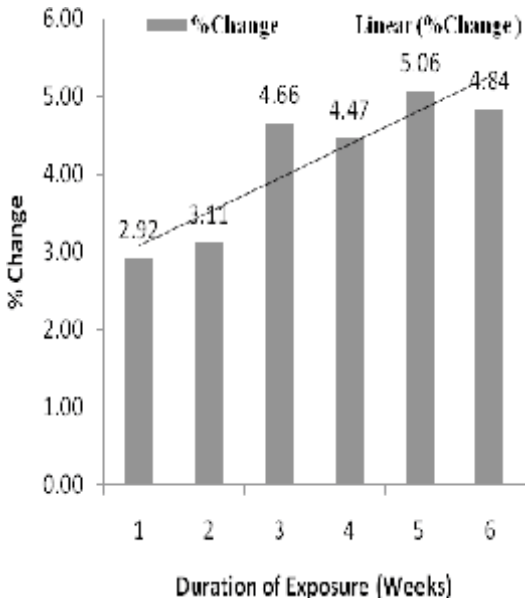


Figure 2. [Left] Percent change in A/G Ratio of *Clarias batrachus* in response to 0.5% coke oven effluent (Long Term Exposure)
Figure 3. [Right] Percent change in Serum Gamma Globulin levels of *Clarias batrachus* in response to 0.5% coke oven effluent (Long Term Exposure)

The data obtained on A/G Ratio from present study as a result of short term and long term exposure agree with Bly *et al.* (1997) that immune functions seem to be suppressed by artificial environmental stress. Declination of A/G Ratio indicates dysfunction of the liver. Exposure to stressors, make the gills leaky to water and ions, that often results in osmo-regulatory imbalance (Mazeoud *et al.*, 1977). Stress due to pollutants resulting in haemodilution may also be the cause of the decrease in A/G Ratio (Jacob *et al.*, 1990).

Earlier workers have reported toxic effects of the coke oven effluent of Bhilai Steel Plant on the various morphological and physiological parameters of fishes. The lethal concentration 50 (LC_{50}) for this effluent by Probit analysis was observed to be 5.16 mg/l for *Channa punctatus* (Mishra and Poddar, 2013a) with significant effects on the hematological indices (TEC, TLC and % Hb) in response to short and long term exposures to its various sublethal concentrations (Mishra and Poddar, 2011, 2013b). Bakde and Poddar (2011) reported depletion in the levels of alkaline and acid phosphatases in gills, liver and gonads of *Cyprinus carpio* in response to 10%, 20% and 30% effluent. In response to similar concentrations, elevations in the ALAT (Alanine amino transferase) and ASAT (Aspartate amino transferase) activities in first week and declines in the third week onwards were also reported by Khewar and Poddar (2013) in *Channa punctatus.*

Table 2a. Summary of ANOVA showing the effect of coke oven effluent of Bhilai Steel Plant on A/G Ratio of *Clarias batrachus* (Concentration = 30%; Duration of Exposure = 6 days).

Source	SS	df	MS	F	*P*
Main Effect					
Group	48.02	1	48.02	13.37	0.002
Error	57.48	8	16.00	03.59	
Total	105.50	17			

Table 2b. Summary of ANOVA showing the effect of coke oven effluent of BSP on Serum Gamma Globulin of *Clarias batrachus* (Concentration = 30%; Duration of Exposure = 6 days).

Source	SS	df	MS	F	*P*
Main Effect					
Group	0.01	1	0.01	196	0.000
Error	8E-04	16	5E-05		
Total	0.01	17			

Table 2c. Summary of ANOVA showing the effect of coke oven effluent of Bhilai Steel Plant on A/G Ratio of *Clarias batrachus* (Concentration = 0.5%; Duration of Exposure = 6 weeks)

Source	SS	df	MS	F	*P*
Time	9.87	5	1.97	1.55	
Group	88.00	1	88.00	69.02	0.000
Time x Group	7.80	5	1.56	1.22	0.309
Error	76.5	60	1.28		
Total	182.17	71			

Table 2d. Summary of ANOVA showing the effect of coke oven effluent of Bhilai Steel Plant on Serum Gamma Globulin of *Clarias batrachus* (Concentration = 0.5%; Duration of Exposure = 6 weeks).

Source	SS	df	MS	F	*P*
Main Effects					
Time	5.74	5	1.15	4.64	0.001
Group	0.01	1	0.01	368.60	0.000
Time x Group	4.63	5	9.25	3.74	0.005
Error	0.00	60	2.47		
Total	0.01	71			

Table 2e. AG Ratio and Serum Gamma Globulin concentration (mg/ml) in *Clarias batrachus* as function of exposure to coke oven effluent (Duncan's multiple range test).

S. No.	Factor - Time	AG Ratio (M ± SE)	Gamma Globulin (M ± SE)
1	Week 1	4.68 ± 0.70^{a}	5.21 ± 0.02^{a}
2	Week 2	4.29 ± 0.68^{a}	5.23 ± 0.02^{a}
3	Week 3	3.98 ± 0.35^{a}	5.27 ± 0.03^{a}
4	Week 4	3.66 ± 0.30^{a}	5.26 ± 0.04^{a}
5	Week 5	3.72 ± 0.31^{a}	5.27 ± 0.03^{b}
6	Week 6	3.69 ± 0.30^{a}	5.29 ± 0.04^{b}
S. No.	Factor - Group	AG Ratio (M ± SE)	Gamma Globulin (M ± SE)
1	Control	2.9 ± 0.08^{a}	5.14 ± 0.00^{a}
2	Test	5.1 ± 0.26^{b}	5.35 ± 0.00^{b}

Means having similar alphabets, as superscripts, are not statistically significant from each other at $p < 0.05$ (Based on Duncan's multiple-range test).

This study further adds information that this effluent possesses the potential for altering the immune response in fishes. As immune response is the result of interaction of innate and adaptive immune system of an organism, thus, it would be reasonable to conclude that acute or chronic exposures to such factors that may lead to stress can predispose fish to infectious diseases. The above finding suggests that the presence of Phenol in aquatic habitats than the recommended value in coke oven effluent of Bhilai Steel Plant has an ability to affect the immune response of *Clarias batrachus* in terms of A/G Ratio and Gamma globulin even in low concentrations. This indicates that the effluent which finally reaches the river Kharoon, although in diluted condition will have deleterious consequences on aquatic organisms living therein.

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